Published in "Annals of the New York Academy of Sciences 1170:
482 - 486, 2009" which should be cited to refer to this work.

The Olfactory Pathway of Adult and Larval *Drosophila*

Conservation or Adaptation to Stage-specific Needs?

Reinhard F. Stocker

Department of Biology, University of Fribourg, Fribourg, Switzerland

Tracing of olfactory projections based on odorant receptor expression has led to an almost complete receptor-to-glomerulus map in adult *Drosophila*. While most of the glomeruli may be involved in processing of food odors, others appear to be more specialized, for example, responding to CO_2 or to pheromonal cues. Recent studies have shed light on signal processing in the antennal lobe and in higher centers. Newly detected cholinergic excitatory local interneurons in the antennal lobe appear to provide substrates for the broad odor tuning properties of projection neurons. In the mushroom bodies, projection neurons establish an intricate divergence-convergence network with their target cells, allowing complex modes of signal transfer. In the lateral horn, projection neurons innervating candidate pheromone glomeruli appear to segregate from those innervating "normal" glomeruli. Hence, pheromone and food information may be handled by separate channels, consistent with discrete behavioral meanings of the two kinds of signals. The olfactory pathway of the larva shares the general layout of its adult counterpart, with a number of simplifications. The presence of only 21 glomeruli suggests a reduction of primary olfactory "dimensions" compared to adults. The existence of a pheromone-sensing subsystem is unlikely. Larval glomeruli are targets of single, unique sensory neurons rather than being sites of convergence as in the adult. Projection neuron outputs are restricted to single glomeruli in the mushroom body. Their target cells either innervate one or several of them creating substrates for elementary odor coding and coincidence detection. In conclusion, olfactory discrimination capacities of the larva are very likely reduced, consistent with the requirements of a substrate feeder.

Key words: Drosophila; **olfaction; adult; larva; antennal lobe; glomeruli; glomerular map; lateral horn; local neurons; mushroom body calyx; olfactory receptor neurons; pheromones; projection neurons**

The discovery of odorant receptors (ORs) in mice[1] and *Drosophila*[2,3] paved the way for dissecting the rules of central olfactory connectivity. Surprisingly, in both species, olfactory receptor neurons (ORNs) usually express a single type of OR, and all ORNs expressing a given OR converge upon a discrete glomerulus in the primary olfactory center.[4–6] Given the reduced numbers of 1300 ORNs, 50 ORs, and 50 antennal lobe glomeruli in adult *Drosophila*,[7,8] this genetic model species is particularly suited for studying the principles of ORN wiring. Indeed, a surprisingly complete OR-to-glomerulus map was recently established (Fig. 1). It reveals a number of interesting properties[8–11]: (1) ORNs in antennae and palps express different ORs and project to different glomeruli, allowing the fly to distinguish between both inputs. (2) Whereas most of the glomeruli may be involved in processing of food odors, some of them appear to accomplish more specialized functions. Two glomeruli are good candidates for mediating pheromonal

Address for correspondence: Reinhard F. Stocker, Department of Biology, University of Fribourg, Chemin du Musée 10, CH-1700 Fribourg, Switzerland. Voice: +41263008875; fax: +41263009741. reinhard.stocker@unifr.ch

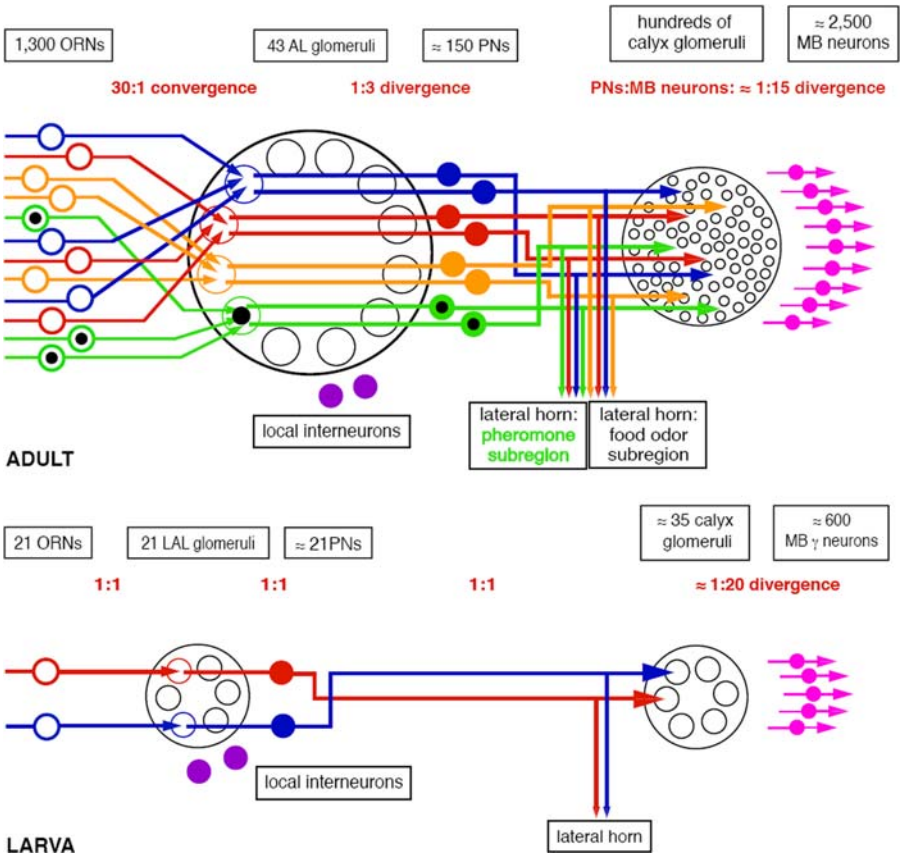

Figure 1. Adult and larval olfactory pathways of *Drosophila* share the same design. However, adults comprise more olfactory receptor neuron (ORN) types (open circles) and more antennal lobe glomeruli. Also, ORNs and projection neurons (PNs; filled circles) exist as multiple copies in the adult, whereas larval ORNs and PNs are unique. Thus, the adult antennal lobe is characterized by converging and diverging connectivity, whereas larval ORNs, antennal lobe glomeruli, PNs, and calyx glomeruli are related essentially in a 1:1:1:1 fashion. Another unique attribute of the adult olfactory pathway is its pheromone-representing subsystem (black dots), a discrete channel that extends all the way from ORNs via antennal lobe glomeruli and PNs to the lateral horn. Adapted from Ramaekers *et al.*[36] with permission from Elsevier. (In color in *Annals* online.)

cues; they are larger in males[12] and they are targets of ORNs that respond to fly odors.[13,14] Another glomerulus comprises the terminals of CO_2-sensitive ORNs[15] and two glomeruli are the targets of putative thermo- or hygrosensensory neurons in the arista.[16] (3) The majority of glomeruli receive bilateral inputs; however, five glomeruli—among them the CO_2 target glomerulus and the aristal glomeruli—are innervated exclusively by the ipsilateral antenna. (4) Target glomeruli of different types of sensilla tend to cluster in different areas of the lobe. (5) At least seven glomeruli are targeted by ORNs expressing two ORs.

The odor signals are processed through local interneurons and projection neurons[10] (Fig. 1). Many of the local interneurons are GABAergic[17]; they establish inhibitory synapses with ORNs and projection neurons. A possible role of this network may be to synchronize projection neuron activity.[18] A second class of cholinergic, excitatory local interneurons[19,20] may allow projection neurons to respond to signals from neighboring glomeruli and may provide

the substrate for the significantly broadened odor tuning of projection neurons compared to ORNs.[18,21] The resulting odor information represented by patterned activity of projection neurons is transferred onto third-order neurons in the mushroom bodies and the lateral horn.[10] The former are key regions for olfactory learning, whereas the lateral horn appears to be involved in naive odor recognition.[22,23] Projection neurons synapse onto multiple mushroom body Kenyon cells and the latter receive input from multiple projection neurons,[24,25] generating a local divergence-convergence network. Hence, Kenyon cells may act as coincidence detectors, which integrate odor information carried by parallel channels of projection neurons.[26,27] In the lateral horn, projection neurons activated by food odors establish stereotypic, but overlapping patterns of terminals.[28,29] They tend to be spatially separated from the terminals of putative pheromone-representing projection neurons which get their inputs from the candidate pheromone glomeruli.[30,31] Pheromone-representing projection neurons establish sexually dimorphic terminal arbors.[32] Thus, information about food appears to become integrated across input channels, whereas pheromones may be signaled via discrete channels all the way to the lateral horn, in accordance with an evolutionarily distinct behavioral meaning of general odors and pheromones. The putative pheromone region receives both excitatory and inhibitory signals[30] which could allow lateral horn neurons to mediate behavioral alternatives, depending on the nature of the pheromone.

The larval olfactory pathway shares the design and the types of neurons of its adult counterpart, but is much simpler in terms of cell numbers[33] (Fig. 1). The merely 21 larval ORNs target single glomeruli, similar to the situation in the adult. However, rather than being sites of ORN convergence, larval glomeruli are targets of single ORNs each expressing its proper OR.[34–36] As in the adult, local interneurons establish interglomerular connections[36] and most of the larval projection neurons focus their dendritic arbors to individual glomeruli.[36,37] However, projection neurons covering more than one glomerulus are common.

Studying the connectivity of projection neurons in the larval mushroom bodies is simplified by the fact that the latter comprise about 30–40 identifiable structures, called calyx glomeruli[36,37] (Fig. 1). Projection neurons choose mostly single calyx glomeruli as targets and many of these neurons stereotypically link a specific antennal lobe glomerulus with a specific calyx glomerulus.[36] Larval Kenyon cells either innervate a single calyx glomerulus[36] or establish arbors in multiple, apparently random glomeruli.[37] Kenyon cells of the two types may allow different modes of signal transfer, that is, elementary odor coding versus coincidence detection.

In conclusion, the olfactory pathway is strongly conserved in larvae and adults, sharing the essential layout of the vertebrate olfactory system (Fig. 1). Yet, the larval circuit displays a number of simplifications, a likely adaptation to simpler olfactory demands. First, every larval ORN and perhaps many of the larval projection neurons are unique.[36] Any loss of these cells should theoretically affect olfactory function more severely than in the adult system. Surprisingly, silencing of single or multiple ORNs has little effect on larval odor-driven behavior, implying that the ligand ranges of the different ORs are largely overlapping.[34] Second, the presence of only 21 antennal lobe glomeruli suggests that the number of primary olfactory "dimensions" is reduced in the larva compared to adult flies comprising about 50 glomeruli. Third, although not studied explicitly, the presence of a pheromone-sensing olfactory subsystem in the larva is unlikely: neither is there any evidence of sex-specific larval behavior nor are any of the known adult pheromone receptors expressed in the larva. Fourth, larval ORN brain projections remain strictly ipsilateral.[33] Finally, given that the numbers of ORNs, antennal lobe glomeruli, projection neurons, and calyx glomeruli are largely similar, the larval olfactory pathway lacks

convergent and divergent connectivity up to the mushroom bodies.[36] This is unlike the adult circuit, in which 1300 ORNs converge onto 50 glomeruli, which diverge again to an estimated 150 projection neurons, each of which innervates many Kenyon cells. The lack of cellular redundancy, the reduced number of primary olfactory dimensions, and the lack of convergent connectivity are likely to reduce the signal-to-noise ratio. Hence, larvae can be expected to perform less well in odor discrimination than adult flies, which may not be a too serious drawback for a substrate feeder.

Acknowledgment

Grant support: Swiss National Funds (3100-A0-105517), Roche Research Foundation.

Conflicts of Interest

The author declares no conflicts of interest.

References

1. Buck, L. & R. Axel. 1991. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* **65:** 175–187.
2. Clyne, P.J., C.G. Warr, M.R. Freeman, *et al*. 1999. A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. *Neuron* **22:** 327–338.
3. Vosshall, L.B., H. Amrein, P.S. Morozov, *et al*. 1999. A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell* **96:** 725–736.
4. Vassar, R., S.K. Chao, R. Sitcheran, *et al*. 1994. Topographic organization of sensory projections to the olfactory bulb. *Cell* **79:** 981–991.
5. Gao, Q., B. Yuan & A. Chess. 2000. Convergent projections of *Drosophila* olfactory neurons to specific glomeruli in the antennal lobe. *Nat. Neurosci.* **3:** 780–785.
6. Vosshall, L.B., A.M. Wong & R. Axel. 2000. An olfactory sensory map in the fly brain. *Cell* **102:** 147–159.
7. Laissue, P.P., C. Reiter, P.R. Hiesinger, *et al*. 1999. Three-dimensional reconstruction of the antennal lobe in *Drosophila melanogaster*. *J. Comp. Neurol*. **405:** 543–552.
8. Couto, A., M. Alenius & B.J. Dickson. 2005. Molecular, anatomical, and functional organization of the *Drosophila* olfactory system. *Curr. Biol.* **15:** 1535–1547.
9. Fishilevich, E. & L.B. Vosshall. 2005. Genetic and functional subdivision of the *Drosophila* antennal lobe. *Curr. Biol.* **15:** 1548–1553.
10. Stocker, R.F. 1994. The organization of the chemosensory system in *Drosophila melanogaster*: a review. *Cell Tiss. Res.* **275:** 3–26.
11. Hallem, E.A. & J.R. Carlson. 2006. Coding of odors by a receptor repertoire. *Cell* **125:** 143–160.
12. Stockinger, P., D. Kvitsiani, S. Rotkopf, *et al*. 2005. Neural circuitry that governs *Drosophila* male courtship behavior. *Cell* **121:** 795–807.
13. Van Der Goes van Naters W. & J.R. Carlson. 2007. Receptors and neurons for fly odors in *Drosophila*. Curr. Biol. **17:** 606–612.
14. Kurtovic, A., A. Widmer & B.J. Dickson. 2007. A single class of olfactory neurons mediates behavioural responses to a *Drosophila* sex pheromone. *Nature* **446:** 542–546.
15. Suh, G.S.B., A.M. Wong, A.C. Hergarden, *et al*. 2004. A single population of olfactory sensory neurons mediates an innate avoidance behavior in *Drosophila*. *Nature* **431:** 854–859.
16. Lienhard, M.C. & R.F. Stocker. 1987. Sensory projection patterns of supernumerary legs and aristae in *D. melanogaster*. *J. Exp. Zool.* **244:** 187–201.
17. Wilson, R.I. & G. Laurent. 2005. Role of GABAergic inhibition in shaping odor-evoked spatiotemporal patterns in the *Drosophila* antennal lobe. *J. Neurosci.* **25:** 9069–9079.
18. Ng, M., R.D. Roorda, S.Q. Lima, *et al*. 2002. Transmission of olfactory information between three populations of neurons in the antennal lobe of the fly. *Neuron* **36:** 463–474.
19. Olsen, S.R., V. Bhandawat & R.I. Wilson. 2007. Excitatory interactions between olfactory processing channels in the *Drosophila* antennal lobe. *Neuron* **54:** 89–103.
20. Shang, Y., A. Claridge-Chang, L. Sjulson, *et al*. 2007. Excitatory local circuits and their implications for olfactory processing in the fly antennal lobe. *Cell* **128:** 601–612.
21. Wilson, R.I., G.C. Turner & G. Laurent. 2004. Transformation of olfactory representations in the *Drosophila* antennal lobe. *Science* **303:** 366–370.
22. De Belle, J.S. & M. Heisenberg. 1994. Associative odor learning in *Drosophila* abolished by chemical ablation of mushroom bodies. *Science* **263:** 692–695.
23. Heimbeck, G., V. Bugnon, N. Gendre, *et al*. 2001. A central neural circuit for experience-independent olfactory and courtship behavior in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **98:** 15336–15341.

24. Tanaka, N.K., T. Awasaki, T. ShimadaT, *et al*. 2004. Integration of chemosensory pathways in the *Drosophila* second-order olfactory centers. *Curr. Biol.* **14:** 449–457.
25. Lin, H.H., J.S. Lai, A.L. Chin, *et al*. 2007. A map of olfactory representation in the *Drosophila* mushroom body. *Cell* **128:** 1205–1217.
26. Heisenberg, M. 2003. Mushroom body memoir: from maps to models. *Nat. Rev. Neurosci.* **4:** 266–275.
27. Perez-Orive, J., O. Mazor, G.C. Turner, *et al*. 2002. Oscillations and sparsening of odor representations in the mushroom body. *Science* **297:** 359–365.
28. Marin, E.C., G.S.X.E. Jefferis, T. Komiyama, *et al*. 2002. Representation of the glomerular olfactory map in the *Drosophila* brain. *Cell* **109:** 243–255.
29. Wong, A.M., J.W. Wang & R. Axel. 2002. Spatial representation of the glomerular map in the *Drosophila* protocerebrum. *Cell* **109:** 229–241.
30. Jefferis, G.S.X.E., C.J. Potter, A.M. Chan, *et al*. 2007. Comprehensive maps of *Drosophila* higher olfactory centers: spatially segregated fruit and pheromone representation. *Cell* **128:** 1187–1203.
31. Schlief, M.L. & R.I. Wilson. 2007. Olfactory processing and behavior downstream from highly selective receptor neurons. *Nat. Neurosci.* **10:** 623–630.
32. Datta, S.R., M.L. Vasconcelos, V. Ruta, *et al*. 2008. The *Drosophila* pheromone cVA activates a sexually dimorphic circuit. *Nature* **452:** 473–477.
33. Python, F. & R.F. Stocker. 2002. Adult-like complexity of the larval antennal lobe of *D. melanogaster* despite markedly low numbers of odorant receptor neurons. *J. Comp. Neurol.* **445:** 374–387.
34. Fishilevich, E., A.I. Domingos, K. Asahina, *et al*. 2005. Chemotaxis behavior mediated by single larval olfactory neurons in *Drosophila*. *Curr. Biol.* **15:** 2086–2096.
35. Kreher, S.A., A.Y. Kwon & J.R. Carlson. 2005. The molecular basis of odor coding in the *Drosophila* larva. *Neuron* **46:** 445–456.
36. Ramaekers, A., E. Magnenat, E.C. Marin, *et al*. 2005. Glomerular maps without cellular redundancy at successive levels of the *Drosophila* larval olfactory circuit. *Curr. Biol.* **15:** 982–992.
37. Masuda-Nakagawa, L.M., N.K. Tanaka & C.J. O'Kane. 2005. Stereotypic and random patterns of connectivity in the larval mushroom body calyx of *Drosophila*. *Proc. Natl. Acad. Sci. USA* **102:** 19027–19032.